IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE HONORABLE BOARD OF PATENT APPEALS AND INTERFERENCES

In re the application of:

Jussi NURMI et al.

Serial Number: 10/579,137 Group Art Unit: 1637

Filed: May 15, 2006 Examiner: Mummert, Stephanie K.

For: NUCLEIC ACID AMPLIFICATION ASSAY AND ARRANGEMENT THEREFOR

APPEAL BRIEF

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REAL PARTY IN INTEREST

An assignment of the invention claimed in this application from the inventors to Abacus Diagnostica Oy, a Finnish corporation, is recorded in the U.S. Patent and Trademark microfilm records at Reel 17,905, Frame 0867. Accordingly, the real party in interest is Abacus Diagnostica Oy.

RELATED APPEALS AND INTERFERENCES

There are no other prior or pending appeals, interferences or judicial proceedings known to Appellants, the Appellants' legal representative, or Abacus Diagnostica Oy which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

STATUS OF CLAIMS

Claims 18, 19 and 21-30 are pending, while claims 1-17 and 20 have been canceled. Each of pending claims 18, 19 and 21-30 is being appealed from the rejection discussed below.

STATUS OF AMENDMENTS AFTER FINAL REJECTION

No amendments have been filed subsequent to the final rejection dated October 12, 2010.

SUMMARY OF CLAIMED SUBJECT MATTER

This invention concerns an improved method for sample purification prior to performing a nucleic acid amplification assay. Such assays require the removal of sample components which inhibit enzymes used in the analysis, interfere with bioaffinity bond formation, increase background signal or otherwise compromise assay performance. Known sample purification techniques, such as extraction of nucleic acids, cell lysis in the presence of chaotropic salts, affinity binding of nucleic acids onto a solid phase, etc., are time-consuming and labor intensive. These sample purification methods are difficult to automate and ill-suited for field conditions (Specification, page 1, line 25 to page 2, line 13).

Sample volume can also be a significant problem. For example, foodstuffs must be tested to ensure they do not contain harmful bacteria such as salmonella or listeria. A food sample, such as cheese or milk, must be concentrated prior to analysis. However, known analyte enrichment techniques, such as physiological enrichment in selective culture media, can require 24-48 hours (Specification, page 2, lines 14-24).

Appellants have discovered a method for quickly and inexpensively purifying and concentrating a sample prior to a nucleic acid amplification assay. The claimed method includes

forcing a sample in a first direction through a filter which retains biological particles,

removing these biological particles from the filter by a flush flow in a second direction opposite the first direction, and

analyzing the biological particles contained in the flush flow by means of a nucleic acid amplification assay,

wherein the flush flow is analyzed for the analyte or analytes without any purification (Specification, page 3, lines 22-29 and original claims 1 and 3).

In one embodiment, an initial filtration is performed which does not retain the biological particles containing the analyte or analytes but retains particles which might interfere with the analysis of the analyte or analytes, the initial filtration being performed prior to forcing said sample in a first direction through a filter which retains the biological particles (Specification, page 9, line 26 to page 10, line 3).

In one embodiment of the assay, retention of the biological particles containing the analyte(s) by the filter is dependent on the size of the particles. In another embodiment, retention of the biological particles is dependent on the chemical properties of the particles (Specification, page 10, lines 16-18).

In one embodiment, the biological particles containing the analyte or analytes are selected from the group consisting of

prokaryotic or eukaryotic cells or spores or components thereof, viruses or viral particles, complexes comprising protein and/or nucleic acid, and any combination thereof (Specification, page 10, lines 10-14. The biological particles can also be bacteria, bacterial cell, plant pollen, mitochondria, chloroplast, cell nuclei, virus, phage, chromosome and ribosome (Specification, page 10, lines 14-15).

The nucleic acid amplification assay may be selected from the group consisting of polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), ligase chain reaction (LCR), proximity ligation assay, nucleic acid sequence based amplification (NASBA), strand displacement amplification (SDA) and any combination thereof (Specification, page 10, lines 21-25).

The flush flow may be a liquid or a gas not contained in the sample. In other embodiments, the biological particles are flushed with the same liquid or gas originally present in the sample (Specification, page 10, lines 25-29).

The analyte may be selected from the group consisting of a living and/or dead cell or virus; a peptide, a protein or complex thereof; a nucleic acid; and any combination thereof. Examples of cells and viruses include a mold, a yeast, a eukaryotic cell or organism, a pathogenic virus and a cancer cell. Examples of

nucleic acids include DNA, RNA and any derivative thereof (Specification, page 10, line 29 to page 11, line 2). Examples of peptides and proteins include a hormone, a growth factor, an enzyme or parts thereof and/or complexes thereof; and any combination thereof.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Claims 18, 19 and 21-30 stand finally rejected under 35 U.S.C. § 102(e) over U.S. Published Application No. 2004/0229349 to Daridon ("Daridon").

ARGUMENT

The Examiner has erroneously interpreted Daridon as requiring analysis of biological particles in a flush flow without any purification. The Examiner has improperly chosen isolated and unrelated disclosures of Daridon and arranged and combined them in accordance with the limitations of the claimed assay.

I. DARIDON FAILS TO DISCLOSE PERFORMING A NUCLEIC ACID AMPLIFICATION ASSAY ON A REVERSE FLUSH FLOW WITHOUT PURIFICATION

Anticipation requires that the four corners of a single, prior art document describe every element of the claimed invention, either expressly or inherently, such that a person of ordinary skill in the art could practice the invention without undue

experimentation, Advanced Display Sys. v. Kent State Univ., 212 F.3d 1272, 54 USPQ2D 1673 (Fed. Cir. 2000).

A. Daridon Does Not Expressly Disclose Its Reverse Flush Flow Should Be Analyzed Without Purification

Independent claim 18 affirmatively requires analysis of biological particles contained in a flush flow by means of a nucleic acid amplification assay, where the flush flow is analyzed for the analyte without any purification. This affirmative "no purification" requirement is nowhere disclosed in Daridon.

The Patent Office relies on a single sentence in Daridon to show the no purification limitation of the claimed assay:

> The right panel of FIG. 18 shows repositioning of retained particle 620 to analysis site 632. Here, site valve 634 is open, but input valves 636 are closed. Particle 620 is displaced from chamber 618, by fluid flowing in reverse across filter channel 616 from waste channel 614, rather than input channel 612. **Since input valves 636 are closed, fluid and particle 620 flow orthogonally to input channel 612, into analysis site 632.** After particle 620 is delivered to analysis site 632, site valve 634 is closed to isolate the particle fluidically from other particles. In other embodiments, additional fluidic lines may be used to deliver reagents to analysis site 632, or analysis site 632 may be a blind channel that is preloaded with such reagents.

Paragraph [0449] (Emphasis added).

Paragraph [0449] explains the "repositioning" of a retained particle to an analysis site in one embodiment of a microfluidic

device which could be combined with other devices to form a microfluidic system. The bolded sentence simply discloses directing the flush flow to a separate analysis site. "Repositioning" does not require that no purification of the flush flow occur prior to analysis. There is simply no disclosure, one way or the other, regarding the flush flow is purified prior to analysis.

One of ordinary skill in the art would not interpret paragraph [0449] as requiring the flush flow be analyzed by analysis site 632 without purification. No information is given as to what type of sample is being analyzed or what type of analysis is performed by analysis site 632. One of ordinary skill in the art would understand purification of the flush flow may be required, depending on the sample and the type of analysis performed.[1] In this regard, immediately preceding paragraph [0448] teaches that an analysis site may be used to "manipulate" the contents of individual particle chambers, which implies the flush flow will be subject to subsequent sample preparation, such as purification, rather than requiring an analysis without any purification.

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[1]Paragraph [0449] is part of Example 5, which describes a microfluidic device for forming and analyzing a particle array using a "cell comb". Paragraph [0453] list applications of such cell combs in "cell assays", but no specific assays are disclosed.

B. Daridon Does Not Inherently Disclose Its Reverse Flush Flow Should Be Analyzed Without Purification

Inherent anticipation requires that the element missing from Daridon *necessarily* would occur or be present. See In re Oelrich, 666 F.2d 578, 212 USPQ 323 (CCPA 1981):

> Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient. [Citations omitted.] If, however, the disclosure is sufficient to show that the natural result flowing from the operation as taught would result in the performance of the questioned function, it seems to be well settled that the disclosure should be regarded as sufficient.

Id. at 581, 212 USPQ at 326.

Daridon's disclosure simply does not show that the natural result flowing from the operation as taught (repositioning of a retained particle to an analysis site) would be an analysis without purification of the flush flow. The specific analysis performed at analysis site 632 is not defined; it can be any type of analysis. Accordingly, one of ordinary skill would not interpret Daridon as *necessarily* performing an analysis of its flush flow without purification.

The Examiner's argument that Daridon affirmatively requires analysis of its reverse flow without purification is *reversible error* under Advanced Display and Oelrich, both supra.

II. DARIDON DOES NOT ARRANGE OR COMBINE ITS PARTICLE RETENTION, REVERSE FLUSH FLOW, AND NUCLEIC ACID AMPLIFICATION DISCLOSURES IN THE SAME WAY AS THE LIMITATIONS OF THE CLAIMED ASSAY

Even assuming, arguendo, that Daridon discloses all the limitations of claim 18, anticipation is not made out unless all of the claim limitations are arranged or combined in the same way as recited in the claim. See Therasense, Inc. v. Becton, Dickinson & Co., 593 F.2d 1325, 93 USPQ2d 1481 (Fed. Cir. 2010), quoting Net MoneyIn, Inc. v. Verisign, Inc., 545 F.3d 1359, 88 USPQ2d 1751 (Fed. Cir. 2008)(Patent claim directed to an internet payment system not anticipated by reference which contained all claim limitations, when two examples had to be combined to produce claimed system). See also Ecolochem, Inc. v. Southern California Edison Co., 227 F.3d 1361, 56 USPQ2D 1065 (Fed. Cir. 2000)(Although reference taught all elements of the claim, it did not contain a discussion suggesting or linking the claim elements and thus did not anticipate claimed invention), and In re Arkley, 455 F.2d 586, 172 USPQ 524 (CCPA 1972)(For anticipation rejection to be proper, "[t]he [prior art] reference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without any need for picking, choosing and combining various disclosures not directly related to each other by the teachings of the cited reference.")

Daridon mentions nucleic acid amplification assays and discloses particle retention and release steps which force a particle in a first direction through a filter which retains the particles, and then remove the particle from the filter by a flush flow in a second direction opposite the first direction. However, Daridon fails to anticipate the claimed assay because the reference does not disclose all of the claim limitations *arranged or combined in the same way as recited in the claim*, Net MoneyIn, supra.

A. Daridon Discloses A Very Large Genus of Microfluidic Particle Analysis Systems

Daridon is a lengthy reference comprising 89 sheets of drawings [Figs. 1-96(c)] and 881 paragraphs of text, including 26 examples, which discloses a very large genus of systems for the manipulation and/or detection of particles [0004]. Indeed, Daridon discloses numerous system elements and mechanisms, and states these elements "may be combined in any suitable order and/or employed for any suitable number of times within a system":

> The invention provides systems, including apparatus, methods, and kits, for the microfluidic manipulation and/or analysis of particles, such as cells, viruses, organelles, beads, **and/or** vesicles. The invention also provides microfluidic mechanisms for carrying out these manipulations and analyses. These mechanisms **may** enable controlled input, movement/ positioning, retention/localization, treatment, measurement, release, and/or output of particles. Furthermore, these mechanisms **may** be

> combined in **any** suitable order **and/or** employed for **any** suitable number of times within a system. Accordingly, these combinations **may** allow particles to be sorted, cultured, mixed, treated, **and/or** assayed, **among others**, as single particles, mixed groups of particles, arrays of particles, heterogeneous particle sets, **and/or** homogeneous particle sets, **among others**, in series **and/or** in parallel. In addition, these combinations **may** enable microfluidic systems to be reused. Furthermore, these combinations **may** allow the response of particles to treatment to be measured on a shorter time scale than was previously possible. Therefore, systems of the invention **may** allow a broad range of cell and particle assays, such as drug screens, cell characterizations, research studies, **and/or** clinical analyses, **among others**, to be scaled down to microfluidic size. Such scaled-down assays **may** use less sample and reagent, **may** be less labor intensive, **and/or may** be more informative than comparable macrofluidic assays.

Daridon, [0119] (emphasis supplied).

Much like the chapters of a textbook, sections of the Daridon disclosure are directed to specific aspects of its microfluidic systems. Each subsection is written to stand independently of other subsections:

I. Microfluidic Systems
   - Definitions and overview [0121-0124]
   - Materials [0125-0127]
   - Methods of fabrication [0128-0133]

II. Physical Structures of Fluid Networks
   - overview [0134-0135]
   - Passages [0136-0140]
   - Reservoirs [0141-0143]
   - Regulators [00144-0146]

III. Particles
   - Overview [0147-0148]
   - Cells [0149-0161]
   - Viruses [0162-0163]

Daridon's examples [0317-0881] illustrate specific embodiments of its microfluidic particle analysis systems. Yet none of them disclose the assay species defined by Appellants' claim 18, in which a biological particle is retained on a filter, and then removed from the filter by a flush flow in a second direction opposite a first direction, followed by analysis of the biological particles contained in the flush flow by means of a nucleic acid amplification assay, where the flush flow is analyzed for the analyte or analytes without any purification.

In short, Daridon provides an extremely broad generic disclosure of microfluidic systems (section I), a broad subgeneric disclosure (sections II-XII) and numerous individual species within each subgenus. Those of ordinary skill are invited to experiment by selecting individual species within these subgenera in the hope they **may** possibly be able to construct a specific system for use in a specific application.

B. Daridon's Nucleic Acid Amplification Assay Disclosures Are Unrelated to Its Particle Retention/Reverse Flow Disclosure

The Patent Office cites paragraphs [0246], [0288] and [0308] of Daridon as disclosing a nucleic acid amplification assay (Official Action mailed October 12, 2010, page 3, lines 13-15). Yet none of these disclosures are arranged or combined with a prior sample purification which employs the particle retention and

reverse flow filtering steps of the claimed assay. Thus, paragraph [0246] discusses detection sites external to the microfluidic system, and includes polymerase chain reaction (PCR) as one of a laundry list of "further manipulations and/or detection methods which may overlap with, but preferably complement, the manipulations and/or methods performed in the microfluidic system". Paragraph [0246] does not directly relate PCR to the specific particle retention and reverse flow filtering steps required in the claimed assay.

Paragraph [0288] lists PCR as one of several genotypic assays which may be conducted on cells in microfluidic systems to measure their genetic constitution:

> Alternatively, or in addition, methods for genotypic assays may include polymerase-mediated amplification of nucleic acids, for example, by thermal cycling (PCR) or by isothermal strand-displacement methods.

Id. There is no mention of sample preparation purification in paragraph [0288], much less a disclosure or suggestion of the specific particle retention and reverse flow filtering steps required in the claimed assay prior to performing a nucleic acid amplification assay.

Paragraph [0308] lists single-cell PCR as one example of single cell assays which may be performed using microfluidic systems:

> Microfluidic systems may be used to perform single-cell assays, which generally comprise any assays that are preferably or necessarily performed on one cell at a time. Examples of single cell assays include patch-clamp analysis, single-cell PCR, single-cell fluorescence in situ hybridization (FISH), subcellular distribution of a protein, and/or differentiation assays (conversion to distinct cell types). In some cases, single-cell assays may be performed on a retained group of two or more cells, by measuring an individual characteristic of one member of the group. In other cases, single-cell assays may require retention of a single cell, for example, when the cell is lysed before the assay.

Id. Although this paragraph mentions certain sample preparation steps such as cell lysis and cell retention, there is no disclosure or suggestion of the specific particle retention and reverse flow filtering steps required in the claimed assay prior to performing a nucleic acid amplification assay.

C. Daridon's Reverse Flow Particle Capture Disclosure is Unrelated to its Nucleic Acid Amplification Assay Disclosure

The Patent Office cites Figs. 17 and 18, and paragraphs [0443-449] of Daridon as disclosing a reverse flow particle capture step and apparatus (Official Action mailed October 12, 2010, page 3, lines 13-15). Yet none of these disclosures include or are arranged or combined with a subsequent nucleic acid amplification assay. Thus, Fig. 17 is "a fragmentary, top plan view of a microfluidic device for forming an array of single particles or groups of particles, in accordance with aspects of the invention"

[0040], while Fig. 18 comprises "a pair of fragmentary, top plan schematic views of a microfluidic device for forming an array of retained particles that may be transferred to an array of separate sites, illustrating particle retention and transfer configurations, on the left and right respectively, in accordance with aspects of the invention" [0041]. There is no drawing reference numeral in either drawing which illustrates or refers to a nucleic acid amplification assay.

Paragraphs [0443-449] are part of Example 5, which illustrates a microfluidic device for forming and analyzing a particle array using a "cell comb". Daridon explains that, in many applications, it is necessary to form an array of cell-analysis chambers, with each chamber containing the same number of cells. These chambers allow multiple experiments, such as drug screens, to be conducted in parallel, in a consistent and comparable fashion [0441]. Nucleic acid amplification assays are not discussed in paragraphs [0443-449].

Paragraph [0449] discloses retaining a particle on a filter, followed by "repositioning" the particle by a reverse flow to an analysis site:

> FIG. 18 shows a device 630 that is similar to device 610, but that includes a separate analysis site 632 opposing each chamber 618. A site valve 634 controls access to analysis site 632, and a pair of input valves 636 isolates each chamber 618 along input

> channel 612. The left panel of FIG. 18 shows a loading configuration for each of valves 634, 636. Here, site valve 634 is closed (indicated by an "X") to prevent input particles 620 from entering analysis site prematurely, and input valves 636 are open to allow particles to access each chamber 618. The right panel of FIG. 18 shows repositioning of retained particle 620 to analysis site 632. Here, site valve 634 is open, but input valves 636 are closed. Particle 620 is displaced from chamber 618, by fluid flowing in reverse across filter channel 616 from waste channel 614, rather than input channel 612. Since input valves 636 are closed, fluid and particle 620 flow orthogonally to input channel 612, into analysis site 632. After particle 620 is delivered to analysis site 632, site valve 634 is closed to isolate the particle fluidically from other particles. In other embodiments, additional fluidic lines may be used to deliver reagents to analysis site 632, or analysis site 632 may be a blind channel that is preloaded with such reagents.

Id. Paragraph [0449] does not disclose whether its analysis site 632 is adapted to perform a nucleic acid amplification assay. In short, there is no disclosure of all of the claim limitations *arranged or combined in the same way as recited in the claim*, Net MoneyIn, supra.

D. The Examiner's Argument that Daridon Links Its Particle Retention/Reverse Flush and Nucleic Acid Amplification Assay Disclosures Is Clearly Erroneous

The Patent Office argues the various paragraphs of Daridon cited in the anticipation rejection as disclosing nucleic acid amplification assays are "necessarily are related to ...microfluidic particle analysis", and thus the claim limitations

are arranged in the same manner as claimed. See page 2, second paragraph, of the Advisory Action. Yet none of the Daridon examples use a microfluidic particle system with nucleic acid amplification. Indeed, the Advisory Action admits Example 5, which illustrates particle retention followed by reverse flush flow to urge the particle to an analysis site, fails to disclose the particular type of analysis to be performed.

Daridon merely discloses that PCR is one of several detection methods which can be used in conjunction with its microfluidic systems (paragraph [0246]). There is no discussion linking PCR and the specific particle retention and reverse flow steps disclosed in paragraph [0449] and Fig. 18 of Daridon.

The Patent Office has picked isolated disclosures of PCR and improperly combined them with specific features of a microfluidic particle analysis system which are not directly related to one another by the teachings of Daridon to construct the claimed assay, Arkley, supra. In short, the Examiner's argument that Daridon discloses all the limitations of the claimed assay arranged in the same manner as claimed is *reversible error* under Net MoneyIn, supra.

CONCLUSION

The legal standard for anticipation is very high, as evidenced by Net MoneyIn, supra. In this case, Daridon simply does not meet the standard for anticipation of Appellants' claims. Instead, the Examiner has committed reversible error by interpreting Daridon to affirmatively require its microfluidic system to analyze particles in a reverse flush flow without purification. Yet another reversible error arose due to her improper combination of Daridon's isolated PCR disclosures of PCR and the specific microfluidic particle analysis device illustrated in Fig. 18 and discussed in paragraph [0449].

Accordingly, this Board is respectfully requested to reverse the anticipation rejection of claims 18, 19 and 21-30 and pass this application on to allowance.

Respectfully submitted,

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CLAIMS APPENDIX

Claims 18, 19 and 21-30

18. (Rejected) A nucleic acid amplification assay for quantitative and/or qualitative analysis of the presence of a specific analyte or specific analytes in a sample, which analytes, if present, are contained in biological particles of said sample, said assay comprising

forcing said sample in a first direction through a filter which retains said biological particles,

removing biological particles from said filter by a flush flow in a second direction opposite said first direction, and

analyzing biological particles contained in said flush flow by means of a nucleic acid amplification assay,

wherein said flush flow is analyzed for the analyte or analytes without any purification.

19. (Rejected) The assay of claim 18, further comprising

performing an initial filtration which does not retain the biological particles containing the analyte or analytes but retains particles which might interfere with the analysis of the analyte or analytes, said initial filtration being performed prior to forcing said sample in a first direction through a filter which retains said biological particles.

21. (Rejected) The assay of claim 18, wherein retention of the biological particles containing the analyte or analytes by said filter is dependent on the size of the particles.

22. (Rejected) The assay of claim 18, wherein retention of the biological particles containing the analyte or analytes by said filter is dependent on the chemical properties of the particles.

23. (Rejected) The assay of claim 18, wherein the biological particles containing the analyte or analytes are selected from the group consisting of prokaryotic or eukaryotic cells or spores or components thereof, viruses or viral particles, complexes comprising protein and/or nucleic acid, and any combination thereof.

24. (Rejected) The assay of claim 23, wherein the biological particles containing the analyte or analytes are selected from the group consisting of bacteria, bacterial cell, plant pollen, mitochondria, chloroplast, cell nuclei, virus, phage, chromosome and ribosome.

25. (Rejected) The assay of claim 18, wherein said nucleic acid amplification assay is selected from the group consisting of

polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), ligase chain reaction (LCR), proximity ligation assay, nucleic acid sequence based amplification (NASBA), strand displacement amplification (SDA) and any combination thereof.

26. (Rejected) The assay of claim 18, wherein said flush flow comprises a liquid or a gas not contained in said sample.

27. (Rejected) The assay of claim 18, wherein the analyte or analytes are selected from the group consisting of a living and/or dead cell or virus; a peptide, a protein or complex thereof; a nucleic acid; and any combination thereof.

28. (Rejected) The assay of claim 27, wherein the analyte or analytes comprises living and/or dead cells and/or viruses selected from the group consisting of a mold, a yeast, a eukaryotic cell or organism, a pathogenic virus and a cancer cell.

29. (Rejected) The assay of claim 27, wherein the analyte or analytes comprises nucleic acids selected from the group consisting of DNA, RNA and any derivative thereof.

30. (Rejected) The assay of claim 27, wherein the analyte or analytes comprises peptides and/or proteins or complexes thereof selected from the group consisting of a hormone, a growth factor, an enzyme or parts thereof and/or complexes thereof; and any combination thereof.

EVIDENCE APPENDIX

1. U.S. Published Application 2004/0229349 to Daridon

This evidence was cited by the Examiner as part of an Official Action mailed March 24, 2010.

RELATED PROCEEDINGS APPENDIX

NONE